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The risk of pre-eclampsia in women taking metformin: systematic review and meta-analysis

Alqudah, A., McKinley, M. C., McNally, R., Graham, U., Watson, C. J., Lyons, T. J., & McClements, L. (2018). The risk of pre-eclampsia in women taking metformin: systematic review and meta-analysis. *Diabetic Medicine*, 35(2), 160-172. <https://doi.org/10.1111/dme.13523>

Published in:
Diabetic Medicine

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
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Full title: The risk of pre-eclampsia in women taking metformin: systematic review and meta-analysis

Running head: Metformin and the risk of pre-eclampsia

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Word count: 4,494 words.

Funding: This study was supported by Queen's University Belfast international PhD funding received from The Hashemite University, Jordan.

Conflicts of Interest: No potential conflicts of interest relevant to this article were reported.

Novelty statement:

- This study demonstrates that metformin \pm insulin treatment is linked to the lower incidence of pre-eclampsia than insulin treatment alone in women with gestational diabetes mellitus (GDM) or Type 2 DM; this is likely associated with reduced weight gain during pregnancy in those taking metformin.
- In other high-risk pregnancies where hypoglycaemic agents are not essential, metformin does not appear beneficial.
- The current clinical guidelines, which recommend insulin treatment as a first line choice in pregnancies complicated by diabetes should be reviewed in light of these findings and adequately designed RCTs with pre-eclampsia as a primary outcome carried are needed.

Abstract

AIMS

Hypertensive disorders in pregnancy, particularly pre-eclampsia, are the leading cause of maternal or foetal morbidity and mortality, and effective treatments are lacking. The aim of this study was to perform meta-analyses of studies evaluating the risk of pre-eclampsia in high-risk insulin-resistant women taking metformin prior to, or during, pregnancy.

METHODS

Medline, EMBASE, Web of Science and Scopus databases were searched. Both randomised controlled trials (RCTs) and prospective observational cohort studies (CSs) of metformin treatment vs. placebo/control or insulin, either prior to, or during, pregnancy were selected. The main outcome measure was the incidence of pre-eclampsia in each treatment group.

RESULTS

Overall, in five RCTs comparing metformin treatment (n=611) to placebo/control (n=609), no difference in the risk of pre-eclampsia was demonstrated (combined/pooled RR=0.86; 95% CI 0.33-2.26; p=0.76; I²=66%). Meta-analysis of four CSs again showed no significant effect (RR=1.21; 95% CI 0.56-2.61; p=0.62; I²=30%). However, a meta-analysis of eight RCTs comparing metformin (n=838) to insulin (n=836) showed reduced risk of pre-eclampsia with metformin (RR=0.68; 95% CI 0.48-0.95; p=0.02; I²=0%). No heterogeneity was present in the metformin vs. insulin analysis of RCTs, whereas high levels of heterogeneity were present in studies comparing metformin to placebo/control. Pre-eclampsia was a secondary outcome in most of the studies. Mean weight gain from enrolment to delivery was lower in the metformin group (p=0.05, metformin vs. placebo; p=0.004, metformin vs. insulin).

CONCLUSIONS

In studies randomising pregnant women to glucose-lowering therapy, metformin is associated with smaller gestational weight gain and a lower risk of pre-eclampsia than insulin.

Introduction

Pre-eclampsia is a complication of pregnancy that occurs in the second half of gestation. It is defined as the new onset, after 20 weeks gestation, of hypertension ($\geq 140/90$ mmHg) and proteinuria (≥ 300 mg per 24 h), or in the absence of proteinuria, any of the following: thrombocytopenia (platelets $< 100,000/\mu\text{l}$), impaired liver function, progressive renal insufficiency, pulmonary oedema or cerebral or visual disturbances[1]. Pre-eclampsia is classified according to gestational age at onset: term pre-eclampsia (onset ≥ 37 weeks), preterm pre-eclampsia (34-37 weeks), and early-onset pre-eclampsia (< 34 weeks)[2]. Severe features of pre-eclampsia include blood pressure $\geq 160/110$ mmHg, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary oedema, cerebral or visual disturbances[1]. Pre-eclampsia is the leading cause of maternal and foetal morbidity and mortality: by conservative estimates, it affects 10 million pregnant women worldwide annually, and is responsible for 76,000 maternal and 500,000 infant deaths[3]. In addition to the short-term risks, pre-eclampsia is associated, later in life, with cardiovascular disease and/or Type 2 diabetes mellitus (Type 2 DM) in both mothers and offspring[4,5].

The incidence of pre-eclampsia is $\sim 4\text{-}6\%$ in the general population, but is greatly increased by insulin-resistant disorders such as gestational diabetes (GDM), Type 2 DM, polycystic ovary syndrome (PCOS) and obesity[6–8]. In women with pre-gestational diabetes, whether Type 1 or Type 2 DM, the risk for pre-eclampsia is increased approximately four-fold[9,10].

There are currently no reliable biomarkers or effective preventative measures, and no treatments for pre-eclampsia other than delivery. The pathogenesis of pre-eclampsia is linked to aberrant angiogenesis and inadequate remodelling of the spiral uterine artery, later leading to the development of an ischemic placenta; however, understanding of underlying mechanisms is inadequate, impeding the rational for development of preventative and treatment strategies.

A few therapeutic approaches have been explored. Low dose aspirin (75 mg daily) may reduce the incidence of pre-eclampsia by up to 25% if taken before 16 weeks gestation[11], and is recommended from 12 weeks gestation for women who have one or more of the following risk factors: history of pre-eclampsia, multifoetal gestation, chronic hypertension, diabetes (Type 1 or 2 DM), renal disease or autoimmune disease (e.g. systemic lupus erythematosus, antiphospholipid syndrome). Anti-oxidant supplements have also been assessed, but so far failed to show benefit in the prevention of pre-eclampsia[10].

Metformin can be safely used in pregnancy[12] enabling investigation of its effects on pregnancy outcomes in women who are at higher risk such as obese women or women with GDM, Type 2 DM, or PCOS[13–16]. Metformin reduces insulin resistance, and mitigates endothelial dysfunction and hyperglycaemia, factors which have been associated with pre-eclampsia[17,18]. Metformin is an AMPK activator, and reduced AMPK pathway activity has also been implicated in the pathogenesis of pre-eclampsia[19,20]. It is established that the circulating anti-angiogenic factor, fms-like tyrosine kinase 1 (sFlt-1) is significantly increased in pregnancies complicated by pre-eclampsia, and recently, it was demonstrated that metformin can reduce sFlt-1 secretion from placental tissue and placental explants[21]. Metformin therefore warrants investigation as a preventive treatment for pre-eclampsia.

The aim of this systematic review and meta-analysis is to evaluate evidence concerning the efficacy of metformin compared to placebo/control or insulin in reducing the incidence of pre-eclampsia in high-risk pregnant women using randomised controlled trials (RCTs) and prospective observational studies.

Methods

Data sources and searches

A systematic literature search was conducted using Medline (1946), Embase (1974), Web of Science, and Scopus databases for eligible studies from inception until November 2016. Filters were not used for the type of study or language, however only studies in human populations were included. In collaboration with the subject librarian (RF) at the Medical Library, Queen's University Belfast, the following terms and keywords were used: a) "Metformin or Glucophage", b) "Pre-eclampsia or Pre-eclamps* or Preeclampsia or Preeclamps*" and c) "Gestational hypertension or Pregnancy-induced hypertension". Combinations of a) AND b) or a) AND c) were also used.

Study inclusion criteria

Only those studies that met all of the following criteria were considered: 1) original study; 2) RCT or prospective observational study/cohort study (CS); 3) women took metformin before pregnancy and/or during pregnancy, and 4) women were followed throughout the pregnancy and pregnancy outcomes were recorded. We only included studies in which pre-eclampsia was diagnosed based on the following criteria **as per guidelines by the American College of Obstetricians and Gynaecologists guidelines [1]**: at least two consecutive blood pressure measures $\geq 140/90$ mm Hg with proteinuria (≥ 0.3 g per 24 hours or 2+ on dipstick testing), with documented onset after gestational week 20. Three studies were included that defined and diagnosed pre-eclampsia in women with new onset hypertension in the absence of proteinuria but with one of the following: haematological involvement, liver involvement, neurological involvement, pulmonary oedema, foetal growth restriction or placental abruption[13,14,22].

Data Extraction

The following data were extracted from the 18 studies selected: study characteristics (author, year of publication, country), population characteristics (age, body mass index (BMI) at enrolment, blood pressure at baseline, weight change during pregnancy, glycaemic control), treatment design (number of women on metformin or placebo/insulin, dose of metformin and duration of treatment) and outcomes (primary and secondary outcomes), Table 1.

Quality Assessment

Quality assessment of the included studies was independently performed by two reviewers (AA and LM) using Critical Appraisal Skills Programme [CASP, c/o Better Value Healthcare Ltd, Oxford] tools specifically designed for RCTs and CSs. Assessment was based on the eleven criteria, with one point being awarded for each if met in the study. The eleven criteria were: 1) clearly focused issue addressed (RCTs and CSs), 2) appropriate randomisation (RCTs) or appropriateness of the method used (CSs), 3) appropriate blinding (RCTs) or recruitment (CSs), 4) matching baseline characteristics (RCTs) or controls selected in an acceptable way (CSs), 5) equal treatment of the groups (RCTs) or biased minimised (CSs), 6) follow-up of the women (RCTs) or adjusted for confounding factors (CSs), 7) the significance of the results (RCTs and CSs), 8) the size effect and precision of the results (RCTs and CSs), 9) ability to apply results locally (RCTs) or how believable the results are (CSs), 10) primary and secondary outcomes (RCTs) or ability to apply results locally (CSs), and 11) whether benefit is worth the harm and costs (RCTs) or whether the results fit other available evidence (CSs). The scores were compared between the two reviewers (AA and LM) and any differences were discussed to reach a consensus.

Assessing the risk of bias was only possible for RCTs. We used RevMan 5.3 (Cochrane, UK) software which automatically generated a panel representing overall risk of bias for each study based on A) random sequence generation, B) allocation and concealment, C) blinding of

participants and personnel, D) blinding outcome assessment, E) incomplete outcome data, F) selective reporting and G) other bias.

Data synthesis and analysis

Risk ratio (RR) and accompanying standard errors were extracted from each study in relation to pre-eclampsia. In each, unadjusted estimates were recorded. A meta-analysis was performed to obtain pooled RR for pre-eclampsia in pregnant women treated with metformin compared with placebo or insulin using RCTs only; for CSs metformin treatment was compared to control (diet control or healthy pregnancies) or insulin. Therefore, four separate analyses were performed to compare effects of metformin vs. placebo/control and metformin vs. insulin, in RCTs only or CSs only, on the incidence of pre-eclampsia. In our analyses, women taking metformin who subsequently needed insulin to maintain good glycaemic control during the course of the pregnancy were included in the metformin arm. Clinical outcomes in RCTs which have shown a positive association with pre-eclampsia (e.g. mean fasting blood glucose (FBG) from enrolment to delivery; HbA1c at 36-37 weeks; GDM incidence; mean weight gain from enrolment to delivery), where available, were used to perform a meta-analysis to obtain pooled standard mean differences in pregnant women treated with metformin compared with placebo or insulin. A meta-analysis was also performed to examine if pooled standard mean differences in age and BMI were different in pregnant women treated with metformin compared with control in CSs.

Random effects models were used to combine estimates to account for any heterogeneity present in the studies. Heterogeneity among studies was tested using a Chi-squared test and measured using the I-squared statistic. RevMan 5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and Stata 12 software (StataCorp LP, College Station, TX) were used to carry out these analyses. First, both RCTs and CSs were

included in the meta-analyses. Following this, only RCTs were included in the meta-analyses. Publication bias was assessed by funnel plots representing the log RR against the standard error[23].

Results

Study selection

As depicted in the PRISMA flow chart (Fig. 1), the database search and literature screening yielded 364 studies. After removing duplicates, two reviewers (AA and LM) screened titles and abstracts of remaining 321 articles. Following initial screening 293 articles were excluded because they did not meet the inclusion criteria and were outside of the scope of the review. Two reviewers (AA and LM) **independently** assessed full text of remaining 28 articles. Three were excluded because they repeated findings from another included study. Three studies did not clearly define pre-eclampsia. Three studies were excluded because corresponding authors did not respond to requests for clarification or additional information[24–26]. **This yielded a total number of 19 studies. The selected studies compared metformin treatment to a healthy cohort of women or a healthy diet (n=5)[22,27–30], or placebo (n=4)[14,16,31,32], or insulin (n=11)[13,15,22,33–40] (one study, with three treatment arms, was included both in metformin vs. control and metformin vs. insulin analysis[22]). The groups of women recruited into selected studies included women with GDM (n=7)[13,15,22,35–37], Type 2 DM (n=1)[34], women with both GDM and Type 2 DM (n=3)[37–39], PCOS (n=6)[16,27–30,32] and obese women (n=2)[14,31]. The quality scores based on the CASP tool assessment for each study are listed in the Supplementary table 1. The main categories where the CSs scored lower include: recruitment, acceptable controls , adjustment for confounding factors and application of the results to the local community. Some RCTs were scored lower in the categories such as**

appropriate blinding (metformin vs. insulin RCTs), the size effect and precision of the results, ability to apply results locally (RCTs) and primary and secondary outcomes.

Study characteristics

Characteristics of the 19 selected studies are described in detail in Table 1. Fourteen RCTs [13–16,30–38,40] and five CSs [22,27–29,39] studies were included. The baseline age range for all the women (n=3,374) was 16-46 years; most of the studies included women less than 35 years old. The majority of women were overweight (BMI: 25-30 kg/m², 35%) or obese (BMI>30 kg/m², 64%); only 1% had a normal BMI at enrollment. Metformin treatment was initiated before pregnancy in three out of 19 studies [27-29], all in women with PCOS, in whom the aim was to enhance fertility. Pre-eclampsia was reported as a primary outcome only in four studies [16,27,29,39] and as a secondary outcome in sixteen studies [13–15,22,28,30–38,40]. All studies were published in or after the year 2000. The studies were carried out worldwide: Australia (n=1); Brazil (n=1); Denmark (n=1); Finland (n=2); Ghana (n=1); Italy (n=1), Iran (n=2); New Zealand (n=2); Norway (n=2); Pakistan (n=2); UK (n=2); USA (n=3).

Meta-analysis of pre-eclampsia incidence

A meta-analysis of five RCTs [14,16,30–32] comparing the effects of metformin vs. placebo included 1,220 pregnant women (611 in the metformin group, and 609 in the placebo group; Fig. 2a). There was no significant difference in the incidence of pre-eclampsia between these two groups; RR in the treatment arm was 0.86 (95% CI 0.33-2.26; p=0.76). There was a significant heterogeneity among these five studies (I²=66%, p=0.02). When the four prospective observational CSs [22,28,29,27] were combined in a meta-analysis, the RR was

1.21 (95% CI 0.56-2.61; $p=0.62$), also demonstrating no difference between metformin vs. control group (diet or no intervention) in terms of pre-eclampsia incidence. Heterogeneity was acceptable at 30%. However, three out of four CSs compared a group of women with PCOS taking metformin vs. a group of healthy pregnant women, which yielded similar pre-eclampsia incidence between the two groups, therefore suggesting possible benefit of metformin in PCOS cohort of pregnant women[27-29].

When the effects of metformin vs. insulin were compared, following exclusion of one RCT[40] in which a high risk of bias was discovered (RevMan 5.3 risk of bias assessment), a meta-analysis of eight RCTs [13,15,33–38] was performed. This meta-analysis included 1,674 pregnant women (838 on metformin treatment and 836 on insulin treatment) and demonstrated a reduction in pre-eclampsia incidence associated with metformin ($RR=0.68$; 95% CI 0.48-0.95, $p=0.02$; Fig. 2c). There was no heterogeneity among these studies in relation to pre-eclampsia incidence ($I^2=0\%$). A meta-analysis of two CSs [22,39] included 956 pregnancies (515 in metformin group and 441 in insulin group) and found no difference in the incidence of pre-eclampsia ($RR=1.61$, 95% CI 0.42-6.12, $p=0.49$, Fig. 2d), however, the results are difficult to interpret due to the small number of studies included and significant heterogeneity between the studies of 79%. All meta-analyses performed, associated forest and funnel plots, and the risk of bias assessment for RCTs are summarized in Fig. 2.

Meta-analyses of clinical characteristics associated with pre-eclampsia

Advanced age, BMI, GDM, glycemic control in women with diabetes and weight gain during pregnancy have all been strongly and independently associated with pre-eclampsia[2,9,17,41–43] and, for this reason, we evaluated if incidence of GDM or glycaemic control and weight

gain status differed between metformin and control or insulin groups, We also explored whether baseline BMI and age differed between groups in CSs.

Metformin vs. control. As the randomisation process in RCTs ensures an even distribution of clinical characteristics between intervention and control groups, the baseline differences in BMI and age in metformin vs control groups was examined in CSs only. A meta-analysis of pooled BMI means from three CSs[27–29] showed a significant difference between metformin and control groups ($p<0.001$, Fig. 3a) and no heterogeneity amongst studies included. This was not surprising as all of these studies used a healthy pregnancy cohort as a control group, therefore suggesting that BMI is a confounding factor in this analysis. When the same analysis was carried out for age three CSs [27-29], no difference was demonstrated between the metformin vs. control group ($p=0.28$, Fig. 3b).

With regard to the incidence of dysglycaemia in PCOS and obese women, no difference was found in the number of GDM cases between metformin and placebo group in RCTs ($n=522$ in metformin group and $n=499$ in placebo group; $RR=0.89$, 95% CI 0.69–1.16, $p=0.4$; Fig. 4a), or between metformin and control group in CSs ($n=714$ in metformin group and $n=888$ in control group; $RR=0.80$, 95% CI 0.39-1.64, $p=0.54$; Fig. 4b). Heterogeneity between the studies was zero with RCTs and 67% with CSs.

Mean weight gain from enrolment to delivery was reported in three RCTs[14,16,31] comparing metformin to placebo and meta-analysis of this data indicated that weight gain was lower in the metformin versus control groups ($p=0.05$; Fig. 4c). However, heterogeneity amongst the studies was very high ($I^2=93\%$) therefore it is difficult to interpret this finding.

Metformin vs. insulin. In relation to glycaemic control we investigated differences in mean FBG and HbA1c between metformin and insulin groups from enrollment until week 36-37. The mean FBG was recorded in six studies[13,15,34,36–38] whereas HbA1c was recorded in five

studies[13,15,33,36,37]. There were no significant differences between metformin and insulin treatment groups in relation to FBG ($p=0.36$; Fig. 5a) and HbA1c ($p=0.73$, mmol/mol, Fig. 5b; $p=0.75$, %, Supplementary Fig. S1). The percentage of women in the metformin group who subsequently received insulin ranged from 14-85% (Supplementary Table S2). The two biggest studies reported that, between them, 33% of women in the metformin groups received supplementary insulin[13,33].

There was substantial variation in the way that weight gain was recorded, so that mean weight gain could only be included from four out of seven studies which included metformin and insulin groups, in the meta-analysis[13,33,36,37]. These studies recorded weight gain from enrolment to delivery; in three studies women were enrolled from approx. 20-34 weeks gestation[13,33,36], whereas one study enrolled women from 10-22 weeks gestations[37]. A meta-analysis of pooled mean weight gain from entry to delivery demonstrated that women on metformin gained less weight during pregnancy than women on insulin ($p=0.004$; Fig. 5c).

High heterogeneity was reported between the studies in relation to the following clinical parameters that were included in the meta-analyses: FBG ($I^2=66\%$), HbA1c ($I^2=78\%$) and mean weight gain ($I^2=78\%$).

Discussion

This is the first systematic review and meta-analysis that focuses on the incidence of pre-eclampsia in pregnant women taking metformin vs. placebo or insulin and elucidates potential clinical mechanisms in insulin-resistant women. Here, we analysed and critically appraised both prospective observational clinical studies and RCTs, which compared the use of metformin treatment with placebo/control or insulin treatment in pregnant women with insulin-resistant disorders such as PCOS, obesity, Type 2 DM and GDM. Such women are at higher

risk of developing complications of pregnancy including pre-eclampsia. We performed meta-analyses separately for CSs and RCTs. We also carried out two separate analyses comparing results for metformin vs. placebo/control and metformin vs. insulin.

Metformin vs. placebo/control. The results obtained suggest that there is no difference in the incidence of pre-eclampsia between women given metformin vs. placebo or control. We recognize that in the RCTs, the presence of a placebo arm implies that a hypoglycaemic agent was not clinically mandated (e.g. PCOS and obese cohorts), and therefore eligible participants are likely to have lesser risk factors than those requiring randomisation to metformin vs. insulin (Type 2 DM and GDM cohorts). Limitations of some studies included comparison of metformin-treated women with PCOS to healthy pregnant controls[27-29]. This was reflected in the meta-analysis of clinical characteristics which found BMI to be higher in metformin than the control group in the CS analysis, and hence is likely to be a confounding factor in relation to risk of pre-eclampsia. Another limitation was that pre-eclampsia was the primary outcome in only four of nine studies: three observational[27,29,39] and one RCT[16]. The heterogeneity between metformin vs. placebo RCTs was also high, therefore the results are difficult to interpret.

Only two studies comparing metformin to placebo recruited obese women with BMI > 30 kg/m² and without diabetes, and, although of similar size, these two studies reached opposite conclusions[14,31]. In one, the number of women with pre-eclampsia was significantly lower in metformin group (odds ratio [OR] = 0.24; p=0.001)[14], whereas in the other, although statistical significance was not reached, a higher incidence of pre-eclampsia was reported in metformin group (OR=2.39; p=0.21)[31]. The baseline characteristics of the participants in both studies were very similar except that one study included all white women[31] whereas the other study included all racial groups and therefore was more representative of the general population in the UK [14]. In the latter study, lower incidence of pre-eclampsia was observed

in the metformin group compared to placebo. In both, pre-eclampsia was recorded as a secondary outcome.

Furthermore, the incidence of GDM was not different between metformin and placebo/control groups whether RCTs or CSs were examined suggesting that metformin did not have a significant effect on preventing GDM. Women who develop GDM have a higher incidence of pre-eclampsia[17], therefore it is possible that metformin in these cohorts of women was unable to prevent GDM (Fig. 4a,b), and, as a result, no difference in pre-eclampsia incidence was observed. On the other hand, weight gain, which was only reported in three RCTs[14,16,31], was borderline significant in favour of metformin being associated with lower weight gain during pregnancy. Therefore, the effect of metformin on weight gain, therefore, appears to be more pronounced in people with DM vs. without DM.

Despite the fact that metformin activates the AMPK pathway, an effect which has been shown to inhibit processes directly relevant to the pathogenesis of pre-eclampsia such as irregular angiogenesis, endothelial dysfunction and inappropriate placental development[19–21], it did not demonstrate superiority over placebo/control in reducing the incidence of pre-eclampsia in this meta-analysis. Interestingly, Vanky and colleagues reported that severe pregnancy complications, which included pre-term delivery before 32 weeks, severe pre-eclampsia or serious post-partum events occurred only in the placebo group (placebo, 7/22 vs. metformin, 0/18, $p=0.01$)[32]. The effect of metformin vs. placebo on severe pre-eclampsia should, therefore, be investigated in the future.

Interestingly, clinical studies which assessed cardiovascular effects of metformin in people without Type 2 DM, showed little or no effect on the markers of cardiovascular disease[44]; the Diabetes Prevention Program also demonstrated no beneficial effect of metformin in reducing the incidence of hypertension in people without Type 2 DM[45]. Conversely, in

people with Type 2 DM the cardiovascular benefits of metformin were well substantiated in the UKPDS trial[46]. This differential effect of metformin in people with vs. without DM could also be relevant to pre-eclampsia, a disease of cardiovascular system, characterised by hypertension and proteinuria.

Metformin vs. Insulin. The comparison between metformin and insulin demonstrated a reduction in RR of pre-eclampsia in favour of metformin in the meta-analysis of RCTs. This result is convincing considering there was no heterogeneity between the studies. Nevertheless, in these studies, a common weakness was that neither the investigators nor the participants were blinded because of the different routes of administration of the study drugs. Glycaemic control was similar between groups at the start or throughout the trial; weight gain after enrolment was significantly lower in metformin group. Weight gain has been linked to an increased risk of pre-eclampsia[47]. Other possibilities for bias included a high risk for random sequence generation, and allocation concealment which was present in two studies[15,34].

Considering all studies, on average 45% of the women in the metformin group needed supplementary insulin (Supplementary Table S2). When we carried out meta-analysis comparing metformin alone vs. insulin alone, which included six RCTs [Supplementary Fig. S2], the incidence of pre-eclampsia remained lower in the metformin group but significance was lost ($p=0.18$, RCTs and CSs; $p=0.21$; RCTs only). Administration of aspirin was not reported in any of the studies included. Nevertheless, most of these studies are relatively small, and therefore there may still be justification for a larger study with pre-eclampsia as a primary outcome, to address the question definitively.

Overall, even though metformin \pm insulin vs. insulin alone was associated with a lower risk for pre-eclampsia, it is unclear whether this is because insulin itself might increase the risk of pre-eclampsia, perhaps in part by causing weight gain, or whether this is a beneficial effect of

metformin. A large population-based registry study in Finland[48] compared pregnancy outcomes in women with GDM who were below or over 35 years of age vs. women without GDM in the same age groups. Women with GDM were treated with diet or insulin. We calculated RR for pre-eclampsia, based on the data presented in the paper, between women with GDM treated on diet vs. insulin treatment in both age groups. This showed that in women with GDM who were below 35 years of age, insulin (238/2845) increased the risk of pre-eclampsia (RR=1.19; CI 1.04 – 1.36; p=0.0092) compared to diet (1161/19422); no difference was found in the prevalence of pre-eclampsia between diet and insulin group in women with GDM who were over 35 years of age. Most of the women (>90%) in our meta-analysis were younger than 35 years, which suggests that metformin may only have a marginal effect on preventing the risk of pre-eclampsia, however, it is still a better option than insulin alone in terms of the risk of pre-eclampsia and possibly other pregnancy complications. Further trials are needed to explore the incidence of pre-eclampsia between insulin and diet interventions. Perhaps prospective studies comparing insulin treatment to diet in women with GDM or Type 2 DM could address this question. It is possible that metformin could have advantages over insulin in pregnant women who require a hypoglycaemic agent; these advantages could be even more pronounced in women over the age of 35 according to the findings by Lamminpää and colleagues[48]. Nevertheless, these women might still need insulin supplementation in the later stages of pregnancy to control hyperglycaemia. In women on metformin ± insulin, the weight gain is less than in women on insulin alone, and it is likely that the dose of insulin may be lower when metformin is used: both factors are potentially beneficial in relation to pre-eclampsia. In contrast, in the studies comparing metformin with placebo or no treatment, hypoglycaemic intervention was either optional or not needed: in these women, the data show no evidence in favour of metformin in reducing the risk of pre-eclampsia. It is important to explore further the effects of metformin vs. placebo/control on the early or severe type of pre-eclampsia

characterised by onset of pre-eclampsia before 34 weeks gestation or blood pressure $\geq 160/110$ mmHg. It is possible, as suggested by Myatt and colleagues, that there are different phenotypes of pre-eclampsia and that this is the reason why large clinical studies have failed to validate findings observed in the smaller studies[49]. Therefore, correct stratification of high-risk women according to age, presence of diabetes, blood pressure or BMI is important and this could determine the most appropriate preventative treatment. Also, women with GDM or Type 2 DM during pregnancy are frequently given, or swapped to, insulin instead of metformin. These women are at high risk of pre-eclampsia; it is possible, based on this review, and other published data, that metformin \pm supplementary insulin would be a better option during pregnancy in these women than insulin alone.

Clearly, in this systematic review we could not include (and did not find) any studies of pregnancy in Type 1 DM women. These women also have a four-fold increased risk of developing pre-eclampsia, similar to women with Type 2 DM[9,10]. Considering that metformin in addition to insulin appears beneficial compared to insulin alone, future randomised double-blind placebo-controlled trials investigating the ability of metformin, in addition to insulin, in prevention of pre-eclampsia in pregnant women with Type 1 DM could be valuable. In the current analysis, with a pre-eclampsia rate of 20% in the insulin group and 14% in the metformin group (estimated based upon a 30% reduction in metformin group observed in this meta-analysis), 650 women with pre-gestational Type 1 DM would need to be recruited in each group to have over 80% power to detect this difference as statistically significant.

Conclusion

In pregnant women requiring hypoglycaemic treatment, metformin alone or metformin in combination with insulin is associated with less weight gain and a lower incidence of pre-eclampsia than insulin alone. This suggests that metformin \pm supplementary insulin treatment is linked to more favourable pregnancy outcomes such as reduced risk of pre-eclampsia than insulin alone. This effect is likely to be age-dependent and associated with reduced weight gain during pregnancy. In other high-risk pregnancies where glucose-lowering agents are not essential, we did not find a case for prescribing metformin. Considering that metformin can safely be used in pregnancy, adequately designed and powered RCTs which have pre-eclampsia as a primary outcome should be carried out in the future in GDM or Type 2 DM, and perhaps in Type 1 DM pregnancies.

Funding: Funding was received from the Hashemite University, Jordan.

Conflict of interest: None reported.

Acknowledgements. The authors thank Dr Chris Cardwell, Centre for Public Health, Queen's University Belfast for his help with RevMan 5.3 software, providing power calculation and for reading the draft and providing feedback. The guarantor for this manuscript is Dr Lana McClements.

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Table 1. Study characteristics and outcomes measured.

Figure 1. PRISMA Guidelines flow diagram.

Figure 2. Meta-analysis comparing the risk ratio of pre-eclampsia in metformin vs. non-metformin treatment group. [A] A meta-analysis of RCTs comparing metformin to placebo. [B] A meta-analysis of cohort studies (CSs) comparing metformin to control group. [C] A meta-analysis of RCTs comparing metformin to insulin. [D] A meta-analysis of CSs comparing metformin to insulin. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.

Figure 3. Meta-analyses of BMI and age in metformin vs. placebo treatment group. [A] A meta-analysis of CSs comparing BMI between metformin and control group. [B] A meta-analysis of CSs comparing age between metformin and control group. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.

Figure 4. Meta-analyses of the incidence of GDM and weight gain in metformin vs. placebo treatment group. [A] A meta-analysis comparing the risk ratio of gestational diabetes (GDM) between metformin and placebo group, RCTs combined. [B] A meta-analysis comparing the risk ratio of GDM between metformin and control group, CSs only. [C] A meta-analysis of weight gain between enrolment and delivery, RCTs combined. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference or risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.

Figure 5. Meta-analyses of clinical risk factors for pre-eclampsia in metformin vs. insulin treatment group, RCTs only. [A] A meta-analysis of mean fasting blood glucose [FBG] from enrolment to delivery. [B] A meta-analysis of glycated haemoglobin [HbA1c, mmol/mol] recorded between 36 and 37 weeks. [C] A meta-analysis of weight gain between enrolment and delivery. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.

Supplementary Table 1. Supplementary Table 2: Study quality assessment using CASP tool.

Supplementary Table 2. Percentage of women in metformin group who were supplemented with additional insulin

Supplementary Fig. 1. Meta-analyses of HbA1c [%] in metformin vs. insulin treatment group, RCTs only. A meta-analysis of glycated haemoglobin [HbA1c] recorded between 36 and 37 weeks. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; standard mean difference was calculated; the overall effect was

measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.

Supplementary Fig. 2. A meta-analysis comparing the risk ratio of pre-eclampsia in metformin only vs. insulin only treatment group, RCTs combined. Random effects models were used to combine estimates and analyses were carried out using RevMan 5.3 software; risk ratio was calculated; the overall effect was measured using Z-test with p values less than 0.05 being statistically significant. Heterogeneity was calculated using Chi² test and measured by I² statistic.